Organisms
Journal of Biological Sciences

REVIEWS

Vol. 1, 2 (DECEMBER 2017)
ISSN: 2532-5876
DOI: 10.13133/2532-5876_2.12

Misperceptions in cell and cancer biology. What pioneers and followers of cell culture bestowed on these fields

Carlos Sonnenschein,[*] Ana M. Soto[*]

[*] *Tufts University School of Medicine, Department of Integrative Physiology and Pathobiology, Boston, USA*

Corresponding author: Carlos Sonnenschein, carlos.sonnenschein@tufts.edu

Abstract

During the period from 1907 to 1912, cell culture pioneers established the basic techniques that ever since have been adopted worldwide by experimental biologists and industry to explore diverse scientific and technological topics. As a result, the knowledge accrued illuminated areas of basic cellular and developmental biology. Inadvertently, however, the cell culture pioneers and their followers also introduced misconceptions that, to this day, have obfuscated progress in the very fields mentioned above. Among the latter, a crucial one has been the adoption of the seldom explicitly mentioned premise that quiescence is the default state of cells in multicellular organisms. This misperception has endured to the present day due to a lack of critical analysis of its relevance and to the lack of an evolutionary perspective by those cell culture pioneers and their followers. Herein, we describe and discuss why and how the above referred misperception took place and has mostly remained unchallenged. A gigantic effort will be needed to remove from the specialized literature and the textbooks the above mentioned misperceptions so new generations of biologists will acquaint themselves with evolutionarily relevant premises on which to base rigorous experimental protocols and interpretation of data.

Keywords: growth factors, carcinogenesis, default state, cell culture, cell cycle

Citation: Sonneschein, C, & Soto, AM, 2017, "Misperceptions in cell and cancer biology. What pioneers and followers of cell culture bestowed on these fields", *Organisms. Journal of Biological Sciences*, vol. 1, no. 2, pp. 83 - 92. DOI: 10.13133/2532-5876_2.12.

1. Introduction

Since the Renaissance, the relentless human quest for knowledge of Nature, and where humans fit into the bewildering complexity of the Earth's environment, began by relying mostly on field observations, the classification of living specimens and an interpretation of the fossil record. Today, despite controversies about the merits of their theories, the impact of Jean Baptiste de Lamarck's and Charles Darwin's contributions figure prominently in how the massive body of biological evidence is interpreted. Some areas in biology, however, deserve scrutiny in order to make the collected evidence better fit changing interpretations of evolutionary theory. One such not-fully appreciated area of inquiry is the impact that cell and tissue culture techniques have had in biology since the beginning of last century, particularly on our current understanding of development and carcinogenesis. This Commentary is aimed at a) providing answers to relevant questions generated by the use and misuse of cell culture as a technological tool in biology, and b) initiating a conversation regarding how to remedy the most obvious damages inflicted on our understanding of biology at large and developmental biology and carcinogenesis in particular. This reassessment requires going over data and interpretations of early events occurring in the field of cell and tissue culture. Preliminary epistemological analyses of this subject have been published elsewhere (Sonnenschein 1999; Sonnenschein 2013; Soto 2016).

2. Beginnings of cell and tissue cultures

Since the early 19th century, biologists who were then called naturalists, accumulated sophisticated details of the structure of living matter observed at different levels of biological organization (simple unicellular or complex multicellular organisms). Beginning with Carolus Linnaeus in the 18th century, the anatomical, histological and cytological description of several living organisms occupied the attention of those naturalists. Among them, was Jean Baptiste de Lamarck, who coined the word *biologie* in order to describe the area covered by the life sciences; he occupies a prominent place in the list of important contributors to the contemporaneous understanding of living beings because he proposed one of the first theories of evolution. The main points of Lamarck's theory were that "(1) nature produced successively all the different forms of life on earth, and (2) environmentally induced behavioral changes lead the way in species change" (Burkhardt 2013). His theory has been, and still is, the object both of praise and criticism. In fact, the re-cent interest in epigenetic inheritance is providing renewed interest and credibility to Lamarckism (Koonin 2009; Koonin 2016). From the 1830s to the 1860s various versions of what would later be known as the cell theory were proposed and during the second half of the 19th century, the light microscope became a crucial tool for describing what was perceived to be the normal and the pathological in development and physiology. In 1859, when Charles Darwin published his influential opus magnum On the Origins of Species, a novel view came to dominate the discourses about evolution. Although Darwin did not then extensively address ontogenetic aspects of organisms, in Chapter 13, he hinted at important basic principles affecting the life cycles of unicellular and multicellular organisms (see below).

Next, we will address just one of those basic principles, namely, control of cell proliferation be-cause it remains a crucial impediment in the interpretation of data aimed at explaining the majority of the subjects mentioned above.

3. Initial steps in cell and tissue culture

German scientists began culturing ex vivo tissues of multicellular organisms during the last two decades of the 19th century. The names Wilhelm Roux and Leo Loeb figure among those pioneers; however their limited success led them to abandon the subject for a while (Landecker 2004). Later, in the first decade of the 20th century, now in the USA, a group of experimentalists revisited the subject and became moderately successful at the practice. Indeed, for over a century now, based on those early attempts, experimental biologists have elucidated subcellular, cellular and developmental processes in multicellular organisms by studying cells and tissues in a fairly simple controlled environment observable initially through transparent glass, and beginning in the 1970s, through plastics. In addition to greatly enriching knowledge in the fields of developmental, cellular and molecular biology, industrial applications of cell and tissue culture techniques have been crucial for the successful manufacture of vaccines, drugs and biological reagents, which in turn in-creased the opportunities to heal and save lives. Un-intentionally and seamlessly however, with those remarkable beneficial outcomes unintended misconceptions were introduced within the experimental biology realm. Here we expose those misconceptions and propose to replace them with robust, reliable, evolutionarily relevant premises that should withstand rigorous epistemological and empirical challenges.

4. A brief historical perspective of tissue culture

What has motivated this exploration of the subject? Our interest in the historical aspects of tissue culture stems from our research interests that, starting in the early 1970s, confronted us with paradoxes in the field of control of cell proliferation. Our re-search program was then centered on explaining how ovarian estrogens affected the proliferation of their target cells. At that time, everyone, including us, was persuaded that estrogens directly stimulated their target cells to proliferate. In fact, cells were believed to be quiescent until they were stimulated by extracellular factors to proliferate. This was what textbooks and research articles then claimed, and incidentally still do (Alberts 2010; Alberts 2014; Weinberg 2014b; Lodish 2000).

Beyond this seemingly narrow interest of ours, there was the biologically fundamental issue of how the proliferation of cells other than those estrogen-target cells we were using was controlled. This has been and still is a basic, fundamental topic in biology at large. After empirically exploring several alter-native hypotheses under the implicit premise that those target cells were directly or indirectly stimulated by estrogens, we concluded, instead, that estrogens do not directly induce the proliferation of their target cells when tested in culture conditions (Soto 1984).

On the contrary, when those same cells were inoculated into animals, they required the administration of estrogens in order to proliferate and form a tumor. Meanwhile, the long-standing evidence that epithelial cells that line the uterine and vaginal mucosa proliferated when estradiol was administered to ovariectomized rodents remained unchallenged. These reproducible though counterintuitive in culture and in animal experimental outcomes represented for us and a few others a genuine paradox (Sonnenschein 1980; Tsai 1991).

In order to resolve such a paradox, we deemed it necessary to re-evaluate the premises that we and most others tacitly accepted in order to design experiments and next to reinterpret the data collected. As mentioned above, our initial narrow interest in how estrogens controlled the proliferation of their target cells related to the much broader context of how the proliferation of cells other than the estrogen-target ones was controlled. In this regard, the key issue to be addressed centered on the proliferative state of cells placed in a suitable environment under defined physicochemical conditions (temperature; CO2 con-centration, etc.) and in the presence of sufficient nutrients. In other words, what was the answer to the question: Is the default state of cells, proliferation or quiescence? Tacitly, those working in this field assumed that the default state of cells in multicellular organisms (metazoa and metaphyta) was quiescence. From an assessment of the data collected in our lab over a period of more than six years, we posited in-stead that a) cells placed in culture conditions exercised their constitutive ability to proliferate, and b) when those cells were inoculated into animals, estrogen administration affected their proliferation status because this hormone cancelled an induced inhibition under which its target cells were actively kept (Soto 1987). Later on, we supported these evaluations by identifying a plasma-borne inhibitor of the proliferation of estrogen-target cells (Sonnenschein 1996). Meanwhile, we also examined the original publications that described growth factors as alleged stimulators of cell proliferation and concluded that the data published by Rita Levi-Montalcini and by Stanley Cohen failed to support the notion that either nerve growth factor or epidermal growth factor directly "stimulated" the proliferation of their alleged target cells (Sonnenschein 1999). Instead, we finally surmised that contrary to the prevailing Zeitgeist, proliferation was the default state of all cells.

In addition to our experimental findings using estrogen-target cells, researchers dealing with the phenomenon of lymphocyte quiescence also found that this is an induced state, namely that proliferation of these cells is actively constrained, i.e., inhibit-ed. Separately, other researchers concluded that embryonic stem cells proliferate constitutively, a phenomenon they called "ground state" (Ying 2008; Leitch 2010; Burdon 2002).

This re-assessment of the available evidence now covering metazoans reconciled the fact that a) unicellular organisms also have proliferation as their own default state, a feature that is axiomatic to microbiologists and is dully acknowledged in some biology textbooks (Luria 1975), and b) this generalization fitted well within an evolutionary perspective because it was difficult to imagine how the early success in the establishment and propagation of cells (bacteria-like organisms) on Earth succeeded if those cells had not been endowed with a dominant and constitutive ability to proliferate (Sonnenschein 1991; Soto 1991; Soto 2016). In this latter regard, our re-interpretation of the published evidence supported the conclusion reached early on by Darwin when he speculated in the On the Origin of Species that "…There is no exception to the rule that every organ-ic being naturally increases at so high a rate, that, if not destroyed, the earth would soon be covered by the progeny of a single pair" (Darwin 1859).

The concept of the default state that we proposed was initially centered on cell proliferation; however, soon we realized that together with proliferation, motility is also a constitutive property of cells and thus, of the default state (Sonnenschein 1999; Soto 2011). Additionally, and consistent with Darwinian theory, each cell division is a source of biological variation because the resulting daughter cells are similar but not identical (Longo 2015). Thus, the updated version of the default state for all cells is proliferation with variation and motility (Soto 2016).

5. Competing outlooks on the control of cell proliferation and the birth of tissue culture

Concluding that proliferation was the default state of all cells represents a significant departure from accepted dogma in biology (Soto 1991). Thus, in order to decide between competing conceptual views, un-avoidable questions begged for plausible answers. For instance, Who generated the misperception that quiescence as the default state was factual, When did such a misperception became part of the Zeitgeist in biology at large? And, finally, Why was such a monumental misperception overlooked by a community of researchers who, for a century, were assiduously using cell and tissue culture as an experimental tool?

Regarding the first question, the published record offers hard evidence of what was done and stated during the period between 1907 and 1915 when researchers began to develop novel experimental techniques under different, at times opposing, tacit or explicit premises. On the one hand, when describing their techniques to grow cells, the pioneers of tis-sue culture, namely Ross G. Harrison, the Montrose Burrows-Alexis Carrel duo and Margaret Reed Lew-is and Warren H. Lewis appear to have been unconcerned with the status of the default state of the cells with which they were working. However, they were implicitly adopting experimental designs and interpreting results under either one or another version of the default state we alluded to above. The record we examined does not clarify whether they discussed the matter among themselves. We therefore conclude that while Harrison remained agnostic regarding whether the default state of the cells he used was either quiescence or proliferation, Burrows and Carrel acknowledged that cells placed in culture flasks became "liberated" from the constrains they were subjected to in the intact animal that they used as primary source of fresh material; this view implied that the default state of their cells was proliferation. Meanwhile, the stated aim of the Lewises strivings was to design a "chemically-defined" culture medium which would have facilitated the identification of "stimulators" of cell proliferation. Under this latter scenario, the implication would have been that for the Lewises the default state of cells was quiescence. For a more detailed analysis of this initial period of the history of cell and tissue culture, see (Sonnenschein 1999; Landecker 2004; Sonnenschein 2013).

Separately and apparently unaware of the pleas of Harrison, the Carrel-Burrows duo and that of the Lewises, Theodor Boveri's narrative, judging by what he wrote in The Origin of Malignant Tumors (1914), oscillated at times between claiming that proliferation was the default state of cells, while quiescence was at others. What is clear is that Boveri pro-posed the idea that cancer is a cell-based disease ("…the problem of tumors is a cell problem" (Bo-veri's italics) (pages 3, 40, 78). And more specifically, Boveri claimed that (a) cancer is a problem of cell proliferation and (b) cancers are due to an abnormal chromosomal re- arrangement ("…cancer is due to a chromosomal rearrangement that eliminates a portion of chromosomal material whose function is to inhibit cell proliferation" pages 26–27, 43–51, 90). Again quoting Boveri, "…in these altered conditions, the [tumor] cell reacts differently to its surroundings and this might be the sole cause of the tendency to unchecked cell multiplication" (page 6). In this latter sentence, Boveri leaves it ambiguous as to how do the "cancer cell" react differently to its surroundings and thus, which default state was he finally proposing.

6. When, where and how was quiescence adopted as the default state of cells in metazoans

Ross Granville Harrison, a noted embryologist, was not interested in the technical aspects of cell culture per se (Oppenheimer 1966; Landecker 2004; Abercrombie 1961; Harrison 1907; Harrison 1910). Instead, Harrison who had previously performed experiments that supported Cajal's theory of nerve fiber development, "invented" tissue culture in or-der to simultaneously test Hensen's syncytial theory of nerve generation and the His-Cajal theory of nerve fiber development that he favored. Harrison succeeded in his aims because the in vitro data supported Cajal's and refuted Hensen's theory (Harrison 1907). Once he settled this issue, for the most part he disappeared from the forefront of tissue culturing and moved on to address other important problems in embryology such as control of organ growth, the morphogenetic field, the emergence of polarity and symmetry in embryonic organs (Abercrombie 1961).

Alexis Carrel and Montrose Burrows used tissue culture techniques to fulfill a different agenda. They expanded on the variety of tissues they cultivated including cancerous ones and in their musings, they stumbled upon the topic of the "stimulation" or "inhibition" of cell proliferation (Carrel 1910; Carrel 1911) but left many of their own questions unanswered. As mentioned above, Carrel explicitly acknowledged that cells in culture were "liberated" and thus were able to achieve this new form of (immortal) life, an idea that readily fits with the premise that proliferation was the default state of those cells (Carrel 1912). Later on, as a tissue culturist, Carrel adopted very meticulous and often over-complicated tissue culture techniques (Abercrombie 1961) aimed at explaining among other topics, senescence and immortality; on this account, he reached rather controversial conclusions (Ebling 1942). In fact, Willmer commented that Carrel's work "caused the method [of tissue culture] to be wrapped up from the beginning in a considerable cocoon of mumbo-jumbo, derived from the practices that were prevalent at the time in the operating theaters of the world." (Witowski 1979; Willmer 1965).

Today, it is acknowledged that serum supple-mentation to a basic mixture of salts, sugars, amino acids and vitamins is necessary to generate maxi-mal cell yields. Serum represents a complex mixture of ions, salts, proteins, sugars, vitamins, hormones, lipids, and a variety of other chemical and physical (osmolarity, etc.) factors that so far have remained mostly unexplored and undefined. Anyways, de-spite the statements of the Carrel-Burrows duo in one sense and those of the Lewises on the other, no clear, unequivocal message emerged from the initial attempts of pioneers of tissue culture and their followers regarding the default state of cells in multi-cellular organisms.

Like the pioneer microbiologists that first succeeded in propagating bacteria in the lab, the Lewises and those that followed them in their quest of a "chemically defined" medium in which metazoan cells could proliferate, operationally called any sub-stance that contributed to the survival and propagation of cells a "growth factor". However, there is a clear epistemological difference between the premises adopted by microbiologists and those of the metazoan cell culture pioneers. The former accept as axiomatic that the default state of unicellular organ-isms is proliferation, and hence that cells are "agents" meaning that they are capable of initiating action (proliferation, movement) (Soto 2016). Instead, the metazoan cell culture experimentalists faced the problem that freshly isolated metazoan cells failed to thrive in their serum-less medium, but mostly propagated well in serum-supplemented media. We now know that the cells' failure to thrive in serum-less culture conditions was not because they were quiescent but because they were dying. The pioneers' interpretation that these cells were quiescent led inevitably to the transformation of the operational concept of "growth factor" to mean a specific "signal" present in serum that induced a passive cell to proliferate. This interpretation, however, does not explain why a dramatic, total reversal of the default state would have emerged with the advent of multicellularity. In other words, what could be the rationale for the passage from a unicellular agent to a passive cell embedded in the organ-ism given that the bulk of the cell cycle components are conserved? Such an evolutionary novelty would require a cogent explanation; to the present, this ex-planation has not been forthcoming. Instead, we propose that the default state of proliferation with variation and motility is still preserved in all organisms. With multicellularity, what emerged were organismal constraints imposed on the cells that limit their ability to proliferate and move.

7. Tissue culture in the 1950s and beyond

A telling comment of the Zeitgeist in the 1950s on growing cells in culture conditions can be gleaned from a quotation from a book chapter written by Charity Waymouth, then a recognized contributor to the field. She wrote "The most promising method of designing media of known composition is first to devise a medium in which the cells survive. When the medium is adequate for prolonged survival, the additional conditions that are necessary to permit growth can be recognized" (Waymouth 1954). Shortly thereafter, the desirability to have chemically-defined media in which metazoan-derived cells could be studied in order to define the why and/or the how cells proliferated was reinforced (Biggers 1957). Under this transparent reductionist approach, cell and tissue culture became crucial methodological tools used by researchers to characterize what were perceived to be "stimulators of cell proliferation."

It was during this period that Rita Levi-Montalcini and Stanley Cohen, at Washington University in St Louis MO, developed their research program that used the words "growth factors" aiming at purifying Nerve Growth Factor (NGF), and Epithelial Growth Factor (EGF), respectively. Our bibliographic search based on published data and quotations by Levi-Montalcini and Cohen verified that neither NGF nor EGF stimulated the proliferation of cells. Equally revealing, to this day, no role on the direct triggering of the proliferation of their alleged target cells has been assigned to either NGF or EGF, or for that matter to other alleged growth factors (Durum 1998). Notwithstanding, these and other alleged growth factors described thereafter helped cement the widely accepted misleading notion that quiescence is the default state of cells in multicellular organisms.

8. Concepts not challenged and questions not asked

As outlined above, two remaining questions are generated from the narrative of the impact of the initial attempts to establish cell and tissue culture technology within cellular and developmental biology research programs with an especial emphasis on cancer research. The first can be formulated as what component of the "big picture" did the pioneers miss in their respective attempts to highlight the contribution of cell culture to experimental biology. The above referred to tissue culture pioneers did not show much concern with the question… why cells proliferated in culture.

Had the pioneers framed this question within an evolutionary perspective, they would likely have found at least two alternatives to choose from. The first one would have been that cells proliferated as soon as they were removed from inhibitory constraints prevalent in the whole intact body. From this perspective, proliferation would have been clearly identified as their default state; Carrel and Burrows were swayed in this direction, but the subject was abandoned thereafter. The second theoretical alternative would have been that once removed from whole organisms in which they were subject to stimulation by hypothesized stimulating "signals", those cells would have become quiescent in culture conditions; thus, the alleged growth factors presumably present in serum would have "stimulated" their proliferation. Imperceptibly but effectively, the second alternative pre-vailed and the operational definition of "growth factors" a la Lewises eventually morphed into the cur-rent meaning of those alleged cell proliferation stimulators.

Under this latter context, the "chemically-defined" media were assumed to provide nutrients that assured the survivability of the cells in culture, while serum would have been the conveyor of alleged stimulators of cell proliferation. This assumption remained unchallenged and the notion that quiescence was the default state of cells in metazoans prevailed. Though not explicitly stated, a 1960 influential paper by Eagle and Piez (Eagle 1960) and subsequent claims made by, among others, Gordon Sato in the 1970s (Sato 1978) served to legitimize the acceptance of quiescence as the default state of cells in multicellular organisms.

It is relevant to mention that methodological shortcomings such as the difficulty in accurately counting cells that grew in culture conditions precluded the rigorous establishment of the growth rate at which cells proliferated. From a historical perspective, accurate cell number estimates in culture were incorporated into routine laboratory practices after Aaron Moscona reintroduced trypsin treatment in the 1950s (following the original observation by Peyton Rous in 1916) as a way to effectively separate cells that tended to stick to one another (Moscona 1952). Also, accurate and reliable particle-counting machines were introduced in the 1960s and 70s. Nonetheless, the resolution of these technical problems did not affect the by then widespread perception that quiescence was the default state of metazoan cells.

9. Carcinogenesis in a flask

Separate from the development of cell culture techniques and starting with Theodor Boveri's 1914 book The Origin of Malignant Tumors, the notion that cancer was due to a faulty control of cell proliferation gained increasing popularity. Arguably, aggressive efforts centered on learning about subcellular aspects of biology following the reductionist approach of the molecular biology revolution. This latter effort was due to the enormous impact generated by the detailed description of the structure of the DNA molecule by Franklin, Collins, Watson and Crick in 1953.

During the second half of the last century, re-search featuring growth factors first (1955-1980) and oncogenes later (1970-2000 and even today) reached their apogee as witnessed by the awarding of four Nobel prizes (two for each subject, respectively) to their discoverers. Most of the popularity that putative growth factors and oncogenes acquired among researchers was pegged to the generous financial resources allocated by the Congress of the United States and invested in the National Institutes of Health under the 1971 declaration of the War on Cancer which was aimed at finding an explanation and an eventual cure for the disease.

10. The impact of initial misperceptions generated by tissue culture on current experimental biology and biomedicine

Aware of the impropriety of adopting here a whiggish historical approach to biology, and with a century of hindsight on what has been productive and unproductive in biology and biomedicine, one may divide the answers to the above-referred to questions into conceptual and pragmatic ones. The conceptual answers would have required that those tissue culture pioneers would have had a solid background in evolutionary theory; in turn, this would have likely prevented them from adopting the premise that quiescence was the default state of cells in metazoans. Such a decision automatically sealed the fate of a strategy that searched for specific observables, in this case, direct stimulators of cell proliferation. The pragmatic answers, on the other hand, deserve an in depth analysis that is beyond the narrow focus of this Commentary. Briefly, however, it should be acknowledged that for technical reasons in the first half of the 20th century neither the pioneers nor their followers could accurately dis-criminate between cell survival, proliferation and quiescence. The technical shortcomings (e.g., lack of accurate cell counting) were

lifted in the second half of the 20th century. Regardless, the adoption of quiescence as the default state of cells in metazoa paved the way for the introduction of misguided experimental approaches that distorted views in fields of biology whose relevance are now being increasingly questioned. The two most obvious are a) the introduction of growth factors and oncogenes (Bishop 1991; Bishop 2003), operational notions that lack re-liable empirical support to explain normal or pathological cell proliferation patterns in metazoan (Sonnenschein 1999; Sonnenschein 2008) and indirectly, b) the acceptance that cancer as a cell-based dis-ease centered on alleged disturbances in the control of cell proliferation.

Researchers siding with the premises of the somatic mutation theory of carcinogenesis (SMT) introduced the notion of oncogene as a dominant mutant gene that, in culture conditions, allegedly over-came the quiescent state and caused unrestrained cell proliferation (Hanahan 2000; Soto 2004). The significance of these alleged crucial participants in carcinogenesis in animals and humans has been seriously challenged even by those who in fact, led the scientific community to adopt them (Bishop 1991; Weinberg 2014a). Recently, mounting lacks of fit between the SMT and data generated while adopting its premises questioned the worthiness of this theory (Sonnenschein 1999; Sonnenschein 2008; Soto 2004). As a result of this reassessment of the cell-based nature of neoplasms, the role of the microenvironment in carcinogenesis and metastases is being increasingly highlighted, while the relevance of mutations that allegedly affect genes (and their gene products) that control the cell cycle is being increasingly muted, but not outright discarded (Weinberg 2014b; Weinberg 2014a; Tomasetti 2017; Nowak 2017). This ad hoc rationalization for the lacks of fit to which we alluded to above amounts to a compromise between the SMT and the TOFT. Such a compromise is unjustifiable because it merges two theories that adopt opposite premises and thus, fails to comprehensively explain the cancer puzzle hardly benefitting either the search for rigorous knowledge or the fate of cancer patients.

11. What might be the impact on cell culture and cancer research of changing the default state of cells from quiescence to proliferation?

The stated aim of this Commentary has been to review the impact of contributions of using cell culture techniques in biology at large and in carcinogenesis in particular for over a century. In order to evaluate the full magnitude of the impact of a switch of the default state of cells from quiescence to proliferation and motility requires a more extended analysis than the one that can be offered here. However, we anticipate that its impact is significant both conceptually and pragmatically. In the practice of cell culture and cancer research, at least two types of changes should occur. First, quiescence as the default state of cells in multicellular organisms should be dismissed. This decision eliminates the search for stimulators of cell proliferation ("growth factors" and "oncogenes") and of motility. And second, by necessity, adopting proliferation with variation and motility as the default state of all cells, should refocus research programs in developmental biology and in Carcinogenesis to search and verify the presence and activity of inhibitory constraints of proliferation and motility operating on cells during morphogenesis. These constraints become less efficient during carcinogenesis ("development gone awry"). Also, by acknowledging that cancer is a tissue-based disease, no compelling argument can then be advanced to justify studying subcellular processes like the cell cycle dynamics as primary research targets to explain the disease; these subcellular processes are shared by both normal and so-called cancer cells (Sonnenschein 2011).

Admittedly, dismissing a role of subcellular components in carcinogenesis does not invalidate studying such components and their interactions as a legitimate research aim in their own right. Instead, we favor following the strategy that concentrates re-search efforts at the level of biological organization where the phenomenon is observed and then move to other levels of organization (upward and down-ward) in order to integrate and complement knowledge of the subject. Moreover, by adopting the notion that cancer is "development gone awry" we prefer to consider cancer as a branch of developmental biology susceptible of being successfully approached experimentally using a tissue-based strategy as suggested by the TOFT.

12. Conclusions

During the period of 1907 to 1915, cell culture pioneers provided the basic elements to apply their experimental method to a wide variety of scientific and technical questions while dealing with the complexity of life in the two-dimensional context of cells attached to a glass or plastic surface. In the last one hundred years, thanks to these contributions, the knowledge generated in several areas of biology, especially in the molecular biology sphere, has been staggering. Inadvertently, however, bootlegged with-in these contributions,

the cell culture pioneers and their followers introduced and later did not challenge important misconceptions resulting from the premises adopted to explain biological process such as the control of cell proliferation during development and cancer. Those pioneers did not make explicit their assumptions about the default state of cells and about the true physiological meaning of growth factors. Though the misunderstandings were generated by those pioneers, what is truly intriguing is the lack of critical appraisal or of challenges to the pioneers' assumptions by researchers who for a century used and improved on these techniques. Despite calls for a reassessment of the subject, the notion that the default state of cells in metazoa is quiescence remains stubbornly unaltered. A gigantic, sustained effort will henceforth be needed to critically assess the specialized literature and the textbooks that carry the above-mentioned misconceptions so that new generations of biologists may be given the opportunity to switch to evolutionarily relevant premises on which to base experimental protocols and interpretation of data.

Acknowledgments

This work was supported by NIH grant ES08314. We are grateful to Cheryl Schaeberle for her excellent editorial assistance. We thank the anonymous reviewers of our manuscript for their helpful suggestions on expanding certain portions of it and constructive comments of their own, some of which have been included in their entirety.

References

Abercrombie, M, 1961, Ross Granville Harrison 1870-1959. *Biographical Memoirs of Fellows of the Royal Society* vol. 7, pag. 111-126.

Alberts, B, 2010, "Model organisms and human health", *Science*, vol. 330, pag. 1724.

Alberts, B, Johnson A, Lewis, J, Morgan, D, Raff, M, Roberts, K, Walter, P, 2014, *Molecular Biology of the Cell.* New York, Garland Science.

Biggers, JD, Rinaldini M, Webb M,1957, *The study of growth factors in tissue culture; The Biological Action of Growth Substances,* Cambridge, Cambridge University Press, pag. 264-297.

Bishop, JM, 1991, "Molecular themes in oncogenesis", *Cell*, vol. 64, pag. 235-248.

Bishop, JM, 2003, *How to Win the Nobel Prize*. Cambridge, Harvard University Press.

Burdon ,T, Smith, A, Savatier, P, 2002, "Signalling, cell cycle and pluripotency in embryonic stem cells", *Trends in Cell Biology*, vol. 12, pag. 432-438.

Burkhardt, RWJ, 2013, "Lamarck, evolution, and the inheritance of acquired characters", *Genetics*, vol. 194, pag.793-805.

Carrel, A, 1912, "On the permanent life of tis-sues outside the body", *J Expt Med*, vol. 15, pag. 516-528.

Carrel, A, Burrows, M, 1910, "Cultivation of sarcoma outside of the body: a second note", *J Am Med Assoc*, vol. 55, pp. 1554.

Carrel, A, Burrows, M, 1911, "Artificial stimulation and inhibition of the growth of normal and sarcomatous tissues". *J Am Med Assoc,* vol. 56, pag. 32-33.

Darwin, C, 1859, *On the Origin of Species*, London, Clowes and Sons.

Durum, SK, Muegge, K, 1998, *Cytokine Knockouts*. Totowa, Humana Press.

Eagle, H, Piez, KA, 1960, "The utilization of proteins by cultured human cells". *J Biol Chem* vol. 235, pag. 1095-1097.

Ebling AH, 1942, "Dr. Carrel's immortal chicken heart: present, authentic facts about the oft-falsified scientific «celebrity»". *Sci Am* Jan. pag. 22-24.

Hanahan, D, Weinberg RA, 2000, "The hallmarks of cancer". *Cell*, vol. 100, pag. 57-70.

Harrison, RG, 1907, "Observations of the living developing nerve fiber". *Anat Rec*, vol. 1, pag. 116-128.

Harrison, RG, 1910, "The outgrowth of the nerve fiber as a mode of protoplasmic movement". *J Exp Zool* vol. 9, pag. 787-846.

Koonin, EV, Wolf, YI, 2009, "Is evolution Darwinian or/and Lamarckian?" *Biol Direct*, vol. 4, pag. 42.

Koonin, EV, Wolf, YI, 2016, "Just how Lamarckian is CRISPR-Cas immunity: the continuum of evolv-ability mechanisms". *Biol Direct vol.* 11, pag. 9.

Landecker, H, 2004, *Culturing Life*. Cambridge, MA, Harvard University Press.

Leitch, HG, Blair, K, Mansfield, W, Ayetey ,H, Humphreys, P, Nichols, J, Surani, MA, &Smith ,A, 2010, "Embryonic germ cells from mice and rats exhibit properties consistent with a generic pluripotent ground state". *Development*, vol. 137, pag. 2279-2287.

Lodish, H, Berk, A, Zipursky, SL, Matsudaira, P, Baltimore, D, Darnell, J, 2000, *Overview of the cell cycle and its control; Molecular Cell Biology*. New York, W.H. Freeman.

Longo, G, Montévil, M, Sonnenschein, C, & Soto, AM, 2015, "In search of principles for a theory of organisms". *J Biosci*, vol. 40, pag. 955-968.

Luria, SE, 1975, *36 Lectures in Biology*. Cambridge, MIT Press.

Moscona A, 1952, "Cell suspensions from organ rudiments of chick embryo". *Exp Cell Res* vol. 3, pag. 535-539.

Nowak MA, Warclaw B, 2017, "Genes, environment, and "bad luck"". *Science*, vol. 355, pag. 1266-1267.

Oppenheimer, J, 1966, "Ross Harrison's contributions to experimental embryology". *Bull Hist Med* vol. 40, pag. 525-543.

Sato G, 1978, "Growth factors and cellular proliferation. Introductory remarks". *Natl Cancer Inst Monogr* vol. 48, pag. 77-79.

Sonnenschein, C, Lee, D, Nguyen, J, Soto, AM, 2013, Unanticipated trends stemming from the history of cell culture: Vitalism in 2012?; in Normandin S, Wolfe C (Eds): *Vitalism and the Scientific Image in Post-Enlightenment Life Science*, 1800-2010 . Springer, pag. 293-310.

Sonnenschein, C, Soto AM, 1980, "But ... are estrogens per se growth-promoting hormones?" *J Nat Cancer Inst* vol. 64, pag. 211-215.

Sonnenschein, C, Soto, AM, 1991, Cell proliferation in metazoans: negative control mechanisms; in Lippman, ME, Dickson, RB (eds): *Regulatory Mechanisms in Breast Cancer*. Boston, MA, Kluwer, pag. 171-194.

Sonnenschein, C, Soto, AM, 1999, *The Society of Cells: Cancer and Control of Cell Proliferation*. New York, Springer Verlag.

Sonnenschein, C, Soto, AM, 2008, "Theories of carcinogenesis: an emerging perspective". *Seminars in Cancer Biology*, vol. 18, pag. 372-377.

Sonnenschein C, Soto AM, 2011, "The death of the cancer cell". *Cancer Res* vol. 71, pag. 4334-4337.

Sonnenschein, C, Soto, AM, Michaelson, CL, 1996, "Human serum albumin shares the properties of estrocolyone-I, the inhibitor of the proliferation of estrogen-target cells". *J Steroid Biochem Molec Biol* vol. 59, pag. 147-154.

Soto AM, Longo, G, Montévil, M, Sonnenschein, C, 2016, "The biological default state of cell proliferation with variation and motility, a fundamental principle for a theory of organisms". *Prog Biophys Mol Biol* vol. 122, pag.16-23.

Soto AM, Sonnenschein, C, 1984, "Mechanism of estrogen action on cellular proliferation: evidence for indirect and negative control on cloned breast tumor cells". *Biochem Biophys Res Commun* vol. 122, pag. 1097-1103.

Soto AM, Sonnenschein, C, 1987, "Cell proliferation of estrogen-sensitive cells: the case for negative control". *Endocr Rev* vol. 8, pag. 44-52.

Soto AM, Sonnenschein, C, 1991, "Regulation of cell proliferation: the negative control perspective". *Ann NY Acad Sci* vol. 628, pag. 412-418.

Soto AM, Sonnenschein, C, 2004 "The somatic mutation theory of cancer: growing problems with the paradigm?" *BioEssays* vol. 26, pag. 1097-1107.

Soto AM, Sonnenschein, C, 2011, "The tissue organization field theory of cancer: A testable replacement for the somatic mutation theory". *Bioessays* vol. 33, pag. 332-340.

Tomasetti C, Li, L, Vogelstein, B, 2017, "Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention". *Science*, vol. 355, pag. 1330-1334.

Tsai, PS, Bern, HA, 1991, "Estrogen-independent growth of mouse vaginal epithelium in organ culture". *J Exp Zool*, vol. 259, pag. 238-245.

Waymouth, C, 1954, "The nutrition of animal cells". *Int Rev Cytol*, vol. 3, pag. 1-68.

Weinberg, RA, 2014a, "Coming full circle-from endless complexity to simplicity and back again". *Cell*, vol. 157, pag. 267-271.

Weinberg, RA, 2014b, *The Biology of Cancer*. New York, Garland Science.

Willmer EN, 1965, *Cells and Tissues in Culture: Methods, Biology, and Physiology*. Cambridge, MA, Academic Press.

Witowski JA, 1979, "Alexis Carrel and the mysticism of tissue culture". *Medical History*, vol. 23, pag. 279-296.

Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, Cohen P, Smith A, 2008, "The ground state of embryonic stem cell self-renewal". *Nature* vol. 453, pag. 519-523.